Title: **SOYBEAN VARIETY 92B84**

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Field of the Invention

10   This invention is in the field of soybean breeding, specifically relating to a soybean variety designated 92B84.

Background of the Invention

The present invention relates to a new and distinctive soybean variety, designated 92B84 which has been the result of years of careful breeding and selection as part of a soybean breeding program. There are numerous steps in the development of any novel, desirable plant germplasm. Plant breeding begins with the analysis and definition of problems and weaknesses of the current germplasm, the establishment of program goals, and the definition of specific breeding objectives. The next step is selection of germplasm that possess the traits to meet the program goals. The goal is to combine in a single variety an improved combination of desirable traits from the parental germplasm. These important traits may include higher seed yield, resistance to diseases and insects, tolerance to drought and heat, and better agronomic qualities.

Field crops are bred through techniques that take advantage of the plant's method of pollination. A plant is self-pollinated if pollen from one flower is transferred to the same or another flower of the same plant. A plant is cross-pollinated if the pollen comes from a flower on a different plant. Soybean plants (*Glycine max*), are recognized to be naturally self-pollinated plants which, while capable of undergoing cross-pollination, rarely do so in nature. Insects are reported by some researchers to carry pollen from one soybean plant to another and it generally is estimated that less than one percent of soybean seed formed in an open planting can be traced to cross-pollination, i.e. less than one percent of soybean seed formed in an open planting is capable of producing F_1 hybrid soybean plants, See Jaycox, "Ecological Relationships between Honey Bees and Soybeans," appearing in the American Bee Journal Vol. 110(8): 306-307 (August 1970). Thus intervention for control of pollination is critical to establishment of superior varieties.

A cross between two different homozygous lines produces a uniform population of hybrid plants that may be heterozygous for many gene loci. A cross of two plants

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each heterozygous at a number of gene loci will produce a population of hybrid plants that differ genetically and will not be uniform. Regardless of parentage, plants that have been self-pollinated and selected for type for many generations become homozygous at almost all gene loci and produce a uniform population of true breeding progeny.

5   Soybeans, (*Glycine max*), can be bred by both self-pollination and cross-pollination techniques. Choice of breeding or selection methods depends on the mode of plant reproduction, the heritability of the trait(s) being improved, and the type of variety used commercially (e.g., F_1 hybrid variety, pureline variety, etc.). For highly heritable traits, a choice of superior individual plants evaluated at a single location will be

10   effective, whereas for traits with low heritability, selection should be based on mean values obtained from replicated evaluations of families of related plants. Popular selection methods commonly include pedigree selection, modified pedigree selection, mass selection, and recurrent selection.

The complexity of inheritance influences choice of the breeding method.

15   Pedigree breeding and recurrent selection breeding methods are used to develop varieties from breeding populations. Pedigree breeding starts with the crossing of two genotypes, each of which may have one or more desirable characteristics that is lacking in the other or which complements the other. If the two original parents do not provide all the desired characteristics, other sources can be included in the breeding population.

20   In the pedigree method, superior plants are selfed and selected in successive generations. In the succeeding generations the heterozygous condition gives way to homogeneous lines as a result of self-pollination and selection. Typically in the pedigree method of breeding five or more generations of selfing and selection is practiced: $F_1 \rightarrow F_2$; $F_2 \rightarrow F_3$; $F_3 \rightarrow F_4$; $F_4 \rightarrow F_5$, etc.

25   Pedigree breeding is commonly used for the improvement of self-pollinating crops. Two parents that possess favorable, complementary traits are crossed to produce an F_1. An F_2 population is produced by selfing one or several F_1's or by intercrossing two F_1's (sib mating). Selection of the best individuals may begin in the F_2 population; then, beginning in the F_3, the best individuals in the best families are

30   selected. Replicated testing of families can begin in the F_4 generation to improve the effectiveness of selection for traits with low heritability. At an advanced stage of inbreeding (i.e., F_6 and F_7), the best lines or mixtures of phenotypically similar lines are tested for potential release as new varieties.

Backcross breeding has been used to transfer genes for simply inherited, highly

35   heritable traits into a desirable homozygous variety or inbred line that is utilized as the recurrent parent. The source of the traits to be transferred is called the donor parent.

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After the initial cross, individuals possessing the desired traits of the donor parent are selected and repeatedly crossed (backcrossed) to the recurrent parent. The resulting plant is expected to have the attributes of the recurrent parent (e.g., variety) and the desirable traits transferred from the donor parent. This approach has been used 5   extensively for breeding disease resistant varieties.

Each soybean breeding program should include a periodic, objective evaluation of the efficiency of the breeding procedure. Evaluation criteria vary depending on the goal and objectives, but should include gain from selection per year based on comparisons to an appropriate standard, overall value of the advanced breeding lines, 10   and number of successful varieties produced per unit of input (e.g., per year, per dollar expended, etc.).

Various recurrent selection techniques are used to improve quantitatively inherited traits controlled by numerous genes. The use of recurrent selection in self-pollinating crops depends on the ease of pollination, the frequency of successful hybrids 15   from each pollination, and the number of hybrid offspring from each successful cross.

Promising advanced breeding lines are thoroughly tested and compared to appropriate standards in environments representative of the commercial target area(s) for three or more years. The best lines are candidates for new commercial varieties; those still deficient in a few traits may be used as parents to produce new populations 20   for further selection.

Publically available or newly-released varieties of soybean can also be used as parental lines or starting materials for breeding or as source populations from which to develop or derive other soybean varieties or breeding lines. These varieties or lines derived from publically available or newly-released varieties can be developed by using 25   breeding methods described earlier, such as pedigree breeding, backcrossing and recurrent selection. As an example, when backcross breeding is used to create these derived lines or varieties in a soybean breeding program, publicly available or newly released varieties of soybeans can be used as a parental line or starting material or source population and can serve as either the donor or recurrent parent. See for 30   example, Fehr, "Breeding Methods for Cultivar Development", Chapter 7, *Soybeans Improvement, Production and Uses*, 2nd ed., Wilcox ed. 1987.

These processes, which lead to the final step of marketing and distribution, can take from eight to twelve years from the time the first cross is made. Therefore, development of new varieties is a time-consuming process that requires precise forward 35   planning, efficient use of resources, and a minimum of changes in direction.

A most difficult task is the identification of individuals that are genetically superior, because for most traits the true genotypic value is masked by other confounding plant traits or environmental factors. One method of identifying a superior plant is to observe its performance relative to other experimental plants and to a widely
5   grown standard variety. Generally a single observation is inconclusive, so replicated observations are required to provide a better estimate of its genetic worth.

In addition to the preceding problem, it is not known how the genotype will react with the environment. This genotype by environment interaction is an important, yet unpredictable, factor in plant breeding. A breeder of ordinary skill in the art cannot
10   predict the genotype, how that genotype will interact with various environments or the resulting phenotypes of the developing lines, except perhaps in a very broad and general fashion. A breeder of ordinary skill in the art would also be unable to recreate the same line twice from the very same original parents, as the breeder is unable to direct how the genomes combine or how they will interact with the environmental
15   conditions. This unpredictability results in the expenditure of large amounts of research resources in the development of a superior new soybean variety.

The goal of soybean breeding is to develop new, unique and superior soybean varieties. In practical application of a chosen soybean breeding program, the breeder initially selects and crosses two or more parental lines, followed by repeated selfing and
20   selection, producing many new genetic combinations. The breeder can theoretically generate billions of different genetic combinations via crossing, selfing and mutations. The breeder has no direct control at the cellular level. Therefore, two breeders will never develop the same line, or even very similar lines, having the same soybean traits.

Each year, the plant breeder selects the germplasm to advance to the next
25   generation. This germplasm is grown under unique and different geographical, climatic and soil conditions, and further selections are then made during and at the end of the growing season. The varieties which are developed are unpredictable for the reasons already mentioned.

Mass and recurrent selections can be used to improve populations of either self-
30   or cross-pollinated crops. A genetically variable population of heterozygous individuals is either identified or created by intercrossing several different parents. The best plants are selected based on individual superiority, outstanding progeny, or excellent combining ability. The selected plants are intercrossed to produce a new population in which further cycles of selection are continued.

35   The single-seed descent procedure in the strict sense refers to planting a segregating population, harvesting a sample of one seed per plant, and using the one-

seed sample to plant the next generation. When the population has been advanced from the F_2 to the desired level of inbreeding, the plants from which lines are derived will each trace to different F_2 individuals. The number of plants in a population declines each generation due to failure of some seeds to germinate or some plants to produce at

5   least one seed. As a result, not all of the F_2 plants originally sampled in the population will be represented by a progeny when generation advance is completed.

In a multiple-seed procedure, soybean breeders commonly harvest one or more pods from each plant in a population and thresh them together to form a bulk. Part of the bulk is used to plant the next generation and part is put in reserve. The procedure

10 has been referred to as modified single-seed descent or the pod-bulk technique.

The multiple-seed procedure has been used to save labor at harvest. It is considerably faster to thresh pods with a machine than to remove one seed from each by hand for the single-seed procedure. The multiple-seed procedure also makes it possible to plant the same number of seeds of a population each generation of

15 inbreeding. Enough seeds are harvested to make up for those plants that did not germinate or produce seed.

Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several reference books (e.g., Allard, 1960; Simmonds, 1979; Sneep et al., 1979; Fehr, 1987).

20 Proper testing should detect major faults and establish the level of superiority or improvement over current varieties. In addition to showing superior performance, there must be a demand for a new variety. The new variety must be compatible with industry standards, or must create a new market. The introduction of a new variety may incur additional costs to the seed producer, the grower, processor and consumer, for special

25 advertising and marketing, altered seed and commercial production practices, and new product utilization. The testing preceding release of a new variety should take into consideration research and development costs as well as technical superiority of the final variety. For seed-propagated varieties, it must be feasible to produce seed easily and economically.

30 Soybean (*Glycine max*), is an important and valuable field crop. Thus, a continuing goal of soybean breeders is to develop stable, high yielding soybean varieties that are agronomically sound. The reasons for this goal are obviously to maximize the amount of grain produced on the land used and to supply food for both animals and humans. To accomplish this goal, the soybean breeder must select and develop

35 soybean plants that have the traits that result in superior varieties.

Pioneer soybean research staff create over 500,000 potential new varieties each year. Of those new varieties, less than 50 and more commonly less than 25 are actually selected for commercial use.

Summary of the Invention

According to the invention, there is provided a novel soybean variety, designated 92B84. This invention thus relates to the seeds of soybean variety 92B84, to the plants of soybean 92B84 and to methods for producing a soybean plant produced by crossing soybean variety 92B84 with itself or another soybean plant, and the creation of variants by mutagenesis or transformation of soybean 92B84. This invention also relates to methods for producing other soybean varieties or breeding lines derived from soybean variety 92B84 and to soybean varieties or breeding lines produced by those methods. Soybean variety 92B84 is characterized by a unique combination of yield potential, disease tolerance, and herbicide resistance for its relative maturity.

Definitions

In the description and tables which follow, a number of terms are used. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided:

B/A = BUSHELS PER ACRE. The seed yield in bushels/acre is the actual yield of the grain at harvest.

BSR = BROWN STEM ROT TOLERANCE. This is a visual disease score from 1 to 9 comparing all genotypes in a given test. The score is based on leaf symptoms of yellowing and necrosis caused by brown stem rot. A score of 9 indicates no symptoms. Visual scores range down to a score of 1 which indicates severe symptoms of leaf yellowing and necrosis.

CNKR = STEM CANKER TOLERANCE. This is a visual disease score from 1 to 9 comparing all genotypes in a given test. The score is based upon premature plant death. A score of 9 indicates no symptoms, whereas a score of 1 indicates the entire experimental unit died very early.

COTYLEDON. A cotyledon is a type of seed leaf. The cotyledon contains the food storage tissues of the seed.

EMBRYO. The embryo is the small plant contained within a mature seed.

F_3. This symbol denotes a generation resulting from the selfing of the F_2 generation along with selection for type and rogueing of off-types. The "F" number is a term commonly used in genetics, and designates the number of the filial generation.

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The "F3" generation denotes the offspring resulting from the selfing or self mating of members of the generation having the next lower "F" number, viz. the F2 generation.

FECL = IRON-DEFICIENCY CHLOROSIS. Plants are scored 1 to 9 based on visual observations. A score of 1 indicates the plants are dead or dying from iron-deficiency chlorosis, a score of 5 means plants have intermediate health with some leaf yellowing and a score of 9 means no stunting of the plants or yellowing of the leaves.

FEY = Frogeye Tolerance. This is a visual disease score from 1 to 9 comparing all genotypes in a given test. The score is based upon leaf lesions. A score of 9 indicates no lesions, whereas a score of 1 indicates severe leaf necrosis.

HABIT. This refers to the physical appearance of a plant. It can be either determinate or indeterminate. In soybeans, indeterminate varieties are those in which stem growth is not limited by formation of a reproductive structure (i.e., flowers, pods and seeds) and hence growth continues throughout flowering and during part of pod filling. The main stem will develop and set pods over a prolonged period under favorable conditions. In soybeans, determinate varieties are those in which stem growth ceases at flowering time. Most flowers develop simultaneously, and most pods fill at approximately the same time.

HGT = Plant Height. Plant height is taken from the top of the soil to top pod of the plant and is measured in inches.

HILUM. This refers to the scar left on the seed which marks the place where the seed was attached to the pod prior to it (the seed) being harvested.

HYPL = HYPOCOTYL ELONGATION. This score indicates the ability of the seed to emerge when planted 3" deep in sand and with a controlled temperature of 25° C. The number of plants that emerge each day are counted. Based on this data, each genotype is given a 1 to 9 score based on its rate of emergence and percent of emergence. A score of 9 indicates an excellent rate and percent of emergence, an intermediate score of 5 indicates average ratings and a 1 score indicates a very poor rate and percent of emergence.

HYPOCOTYL. A hypocotyl is the portion of an embryo or seedling between the cotyledons and the root. Therefore, it can be considered a transition zone between shoot and root.

LDG = LODGING RESISTANCE. Lodging is rated on a scale of 1 to 9. A score of 9 indicates erect plants. A score of 5 indicates plants are leaning at a 45° angle in relation to the ground and a score of 1 indicates plants are laying on the ground.

LEAFLETS. These are part of the plant shoot, and they manufacture food for the plant by the process of photosynthesis.

LLE = Linoleic Acid Percent. Linoleic acid is one of the five most abundant fatty acids in soybean seeds. It is measured by gas chromatography and is reported as a percent of the total oil content.

LLN = Linolenic Acid Percent. Linolenic acid is one of the five most abundant fatty acids in soybean seeds. It is measured by gas chromatography and is reported as a percent of the total oil content.

MAT ABS = ABSOLUTE MATURITY. This term is defined as the length of time from planting to complete physiological development (maturity). The period from planting until maturity is reached is measured in days, usually in comparison to one or more standard varieties. Plants are considered mature when 95% of the pods have reached their mature color.

MATURITY GROUP. This refers to an agreed-on industry division of groups of varieties, based on the zones in which they are adapted primarily according to day length or latitude. They consist of very long day length varieties (Groups 000, 00, 0), and extend to very short day length varieties (Groups VII, VIII, IX, X).

OIL = Oil Percent. Soybean seeds contain a considerable amount of oil. Oil is measured by NIR spectrophotometry, and is reported on an as is percentage basis.

OLC = OLEIC ACID PERCENT. Oleic acid is one of the five most abundant fatty acids in soybean seeds. It is measured by gas chromatography and is reported as a percent of the total oil content.

PLM = Palmitic Acid Percent. Palmitic acid is one of the five most abundant fatty acids in soybean seeds. It is measured by gas chromatography and is reported as a percent of the total oil content.

POD. This refers to the fruit of a soybean plant. It consists of the hull or shell (pericarp) and the soybean seeds.

PRT = PHYTOPHTHORA TOLERANCE. Tolerance to Phytophthora root rot is rated on a scale of 1 to 9, with a score of 9 being the best or highest tolerance ranging down to a score of 1 which indicates the plants have no tolerance to Phytophthora.

PRM = Predicted Relative Maturity. Soybean maturities are divided into relative maturity groups. In the United States the most common maturity groups are 0 through VIII. Within these maturity groups the industry generally divides maturities into tenths of a relative maturity group. Within narrow comparisons, the difference of a tenth of a relative maturity group equates very roughly to a day difference in maturity at harvest.

PRO = Protein Percent. Soybean seeds contain a considerable amount of protein. Protein is generally measured by NIR spectrophotometry, and is reported on an as is percentage basis.

PUBESCENCE. This refers to a covering of very fine hairs closely arranged on the leaves, stems and pods of the soybean plant.

RKA = Root-knot nematode, peanut. This is a visual disease score from 1 to 9 comparing all genotypes in a given test. The score is based upon digging plants to look

5   at the roots for presence or absence of galling. A score of 9 indicates that there is no galling of the roots, a score of 1 indicates large severe galling cover most of the root system which results in pre-mature death from decomposing of the root system.

RKI = Root-knot nematode, southern. This is a visual disease score from 1 to 9 comparing all genotypes in a given test. The score is based upon digging plants to

10   visually score the roots for presence or absence of galling. A score of 9 indicates that there is no galling of the roots, a score of 1 indicates large severe galling cover most of the root system which results in pre-mature death from decomposing of the root system.

SDS = Sudden Death Syndrome. Tolerance to Sudden Death Syndrome is rated on a scale of 1 to 9, with a score of 1 being very susceptible ranging up to a score of 9

15   being resistant.

S/LB = Seeds per Pound. Soybean seeds vary in seed size, therefore, the number of seeds required to make up one pound also varies. This affects the pounds of seed required to plant a given area, and can also impact end uses.

SH = SHATTERING. This refers to the amount of pod dehiscence prior to

20   harvest. Pod dehiscence involves seeds falling from the pods to the soil. This is a visual score from 1 to 9 comparing all genotypes within a given test. A score of 9 means pods have not opened and no seeds have fallen out. A score of 5 indicates approximately 50% of the pods have opened, with seeds falling to the ground and a score of 1 indicates 100% of the pods are opened.

25   SHOOTS. These are a portion of the body of the plant. They consist of stems, petioles and leaves.

STC = STEARIC ACID PERCENT. Stearic acid is one of the five most abundant fatty acids in soybean seeds. It is measured by gas chromatography and is reported as a percent of the total oil content.

30   WH MD = WHITE MOLD TOLERANCE. This is a visual disease score from 1 to 9 comparing all genotypes in a given test. The score is based upon observations of mycelial growth and death of plants. A score of 9 indicates no symptoms. Visual scores of 1 indicate complete death of the experimental unit.

Detailed Description of the Invention

A soybean variety needs to be highly homogeneous, homozygous and reproducible to be useful as a commercial variety. There are many analytical methods available to determine the homozygotic and phenotypic stability of these inbred lines.

5   The oldest and most traditional method of analysis is the observation of phenotypic traits. The data is usually collected in field experiments over the life of the soybean plants to be examined. Phenotypic characteristics most often observed are for traits associated with seed yield, seed protein and oil content, lodging resistance, disease resistance, maturity, plant height , and shattering.

10   In addition to phenotypic observations, the genotype of a plant can also be examined. There are many laboratory-based techniques available for the analysis, comparison and characterization of plant genotype; among these are Isozyme Electrophoresis, Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNAs (RAPDs), Arbitrarily Primed Polymerase Chain Reaction

15   (AP-PCR), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs), Amplified Fragment Length Polymorphisms (AFLPs), and Simple Sequence Repeats (SSRs) which are also referred to as Microsatellites.

The variety of the invention has shown uniformity and stability for all traits, as described in the following variety description information. It has been self-pollinated a

20   sufficient number of generations, with careful attention to uniformity of plant type to ensure homozygosity and phenotypic stability. The line has been increased with continued observation for uniformity. No variant traits have been observed or are expected in 92B84, as described in Table 1 (Variety Description Information).

Soybean variety 92B84 is a white flowered, soybean variety with tawny

25   pubescence, black hila, and a relative maturity of 28. Variety 92B84 also demonstrates very good yield in combination with Multi-race Phytophthora resistance (Rps1k). 92B84 further demonstrates resistance to Brown Stem Rot and moderate Sudden Death Syndrome tolerance. Variety 92B84 further demonstrates above average iron deficiency chlorosis tolerance and displays a substantial degree of glyphosate resistance. Variety

30   92B84 is particularly suited to the North Plains, Plains, Southern Plains and Eastern regions of the United States including Iowa, Illinois, Indiana, Missouri, Michigan, Wisconsin and Ohio. This variety does well in *Phytophthora megasperma* infected soils where the Rps1k gene provides resistance. Fields with a history of white mold should be avoided with this variety.

35   Soybean variety 92B84, being substantially homozygous, can be reproduced by planting seeds of the line, growing the resulting soybean plants under self-pollinating or

sib-pollinating conditions, and harvesting the resulting seed, using techniques familiar to the agricultural arts.

TABLE 1

VARIETY DESCRIPTION INFORMATION
92B84

A. Mature Seed Characteristics:
    Seed Coat Color: yellow
    Seed Size (grams per 100 seeds): 15
    Hilum Color: black

B. Leaf:
    Leaflet Shape: ovate
    Leaf Color: medium green

C. Plant Characteristics:
    Flower Color: white
    Pod Color: brown
    Plant Pubescence Color: tawny
    Plant Habit: indeterminate
    Maturity Group: II
    Maturity Sub-Group: 8

D. Fungal Diseases (S = susceptible R = resistant)
    Brown Stem Rot (*Phialophora gregata*): outstanding resistance
    Phytophthora Root Rot (*Phytophthora megasperma* var. *sojae*):
        Race 1: R   Race 4: R   Race 5: R
        Race 7: R

E. Nematode Diseases (S = susceptible R = resistant)
    Soybean Cyst Nematode
        Race 3: S
    Soybean Cyst Other Specify: Race 14: S
    Iron Chlorosis: moderate tolerance

F. Submitted Seed Content (% Protein): 35
    Submitted Seed Content (% Oil): 20

Publications useful as references in interpreting Table1 include:
Caldwell, B. E. ed. 1973. "Soybeans: Improvement, Production, and Uses" Amer. Soc. Agron. Monograph No. 16;
Hartman, G.L., J.B. Sinclair, and J.C. Rupe, 1999. "Compendium of Soybean Diseases, Fourth Edition" The American Phytopathological Soc.
Buttery, B. R., and R. I. Buzzell 1968. "Peroxidase Activity in Seed of Soybean Varieties" Crop Sci. 8: 722-725;
Hymowitz, T. 1973. "Electrophoretic analysis of SBTI-A2 in the USDA Soybean Germplasm Collection" Crop Sci., 13: 420-421;

Payne R. C., and L.F. Morris, 1976. "Differentiation of Soybean Varieties by Seedling Pigmentation Patterns" J. Seed. Technol. 1: 1-19. The disclosures of which are each incorporated by reference in their entirety.

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Further Embodiments of the Invention

TRANSFORMATION OF SOYBEAN

10   With the advent of molecular biological techniques that have allowed the isolation and characterization of genes that encode specific protein products, scientists in the field of plant biology developed a strong interest in engineering the genome of plants to contain and express foreign genes, or additional, or modified versions of native, or endogenous, genes (perhaps driven by different promoters) in order to alter

15   the traits of a plant in a specific manner. Such foreign, additional and/or modified genes are referred to herein collectively as "transgenes". Over the last fifteen to twenty years several methods for producing transgenic plants have been developed, and the present invention, in particular embodiments, also relates to transformed versions of the claimed variety or line.

20   Plant transformation involves the construction of an expression vector which will function in plant cells. Such a vector comprises DNA comprising a gene under control of or operatively linked to a regulatory element (for example, a promoter). The expression vector may contain one or more such operably linked gene/regulatory element combinations. The vector(s) may be in the form of a plasmid, and can be used

25   alone or in combination with other plasmids, to provide transformed soybean plants, using transformation methods as described below to incorporate transgenes into the genetic material of the soybean plant(s).

Expression Vectors For Soybean Transformation
30   **Marker Genes**

Expression vectors include at least one genetic marker, operably linked to a regulatory element (a promoter, for example) that allows transformed cells containing the marker to be either recovered by negative selection, *i.e.* inhibiting growth of cells that do not contain the selectable marker gene, or by positive selection, *i.e.*, screening

35   for the product encoded by the genetic marker. Many commonly used selectable marker genes for plant transformation are well known in the transformation arts, and include, for example, genes that code for enzymes that metabolically detoxify a selective

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chemical agent which may be an antibiotic or a herbicide, or genes that encode an altered target which is insensitive to the inhibitor. A few positive selection methods are also known in the art.

One commonly used selectable marker gene for plant transformation is the neomycin phosphotransferase II *(nptII)* gene, isolated from transposon Tn5, which when placed under the control of plant regulatory signals confers resistance to kanamycin. Fraley *et al., Proc. Natl. Acad. Sci. U.S.A.,* 80: 4803 (1983). Another commonly used selectable marker gene is the hygromycin phosphotransferase gene which confers resistance to the antibiotic hygromycin. Vanden Elzen *et al., Plant Mol. Biol.,* 5: 299 (1985).

Additional selectable marker genes of bacterial origin that confer resistance to antibiotics include gentamycin acetyl transferase, streptomycin phosphotransferase, aminoglycoside- 3′ -adenyl transferase, the bleomycin resistance determinant. Hayford *et al., Plant Physiol.* 86: 1216 (1988), Jones *et al., Mol. Gen. Genet.,* 210: 86 (1987), Svab *et al., Plant Mol.. Biol.* 14: 197 (1990), Hille *et al., Plant Mol. Biol.* 7: 171 (1986). Other selectable marker genes confer resistance to herbicides such as glyphosate, glufosinate or broxynil. Comai *et al., Nature* 317: 741-744 (1985), Gordon-Kamm *et al., Plant Cell* 2: 603-618 (1990) and Stalker *et al., Science* 242: 419-423 (1988).

Other selectable marker genes for plant transformation are not of bacterial origin. These genes include, for example, mouse dihydrofolate reductase, plant 5 -*eno*lpyruvylshikimate-3 -phosphate synthase and plant acetolactate synthase. Eichholtz *et al., Somatic Cell Mol. Genet.* 13: 67 (1987), Shah *et al., Science* 233: 478 (1986), Charest *et al., Plant Cell Rep.* 8: 643 (1990).

Another class of marker genes for plant transformation require screening of presumptively transformed plant cells rather than direct genetic selection of transformed cells for resistance to a toxic substance such as an antibiotic. These genes are particularly useful to quantify or visualize the spatial pattern of expression of a gene in specific tissues and are frequently referred to as reporter genes because they can be fused to a gene or gene regulatory sequence for the investigation of gene expression. Commonly used genes for screening presumptively transformed cells include β-glucuronidase *(GUS),* β-galactosidase, luciferase and chloramphenicol acetyltransferase. Jefferson, R.A., *Plant Mol. Biol. Rep.* 5: 387 (1987)., Teeri *et al., EMBO J.* 8: 343 (1989), Koncz *et al., Proc. Natl. Acad. Sci. U.S.A.* 84:131 (1987), De Block *et al., EMBO J.* 3: 1681 (1984).

Recently, *in vivo* methods for visualizing GUS activity that do not require destruction of plant tissue have been made available. Molecular Probes Publication

2908, Imagene Green™, p. 1-4 (1993) and Naleway *et al.*, *J. Cell Biol.* 115: 15la (1991). However, these *in vivo* methods for visualizing GUS activity have not proven useful for recovery of transformed cells because of low sensitivity, high fluorescent backgrounds and limitations associated with the use of luciferase genes as selectable markers.

5   More recently, a gene encoding Green Fluorescent Protein (GFP) has been utilized as a marker for gene expression in prokaryotic and eukaryotic cells. Chalfie *et al.*, *Science* 263: 802 (1994). GFP and mutants of GFP may be used as screenable markers.

10   **Promoters**

Genes included in expression vectors must be driven by a nucleotide sequence comprising a regulatory element, for example, a promoter. Several types of promoters are now well known in the transformation arts, as are other regulatory elements that can be used alone or in combination with promoters.

15   As used herein "promoter" includes reference to a region of DNA upstream from the start of transcription and involved in recognition and binding of RNA polymerase and other proteins to initiate transcription. A "plant promoter" is a promoter capable of initiating transcription in plant cells. Examples of promoters under developmental control include promoters that preferentially initiate transcription in certain tissues, such

20   as leaves, roots, seeds, fibers, xylem vessels, tracheids, or sclerenchyma. Such promoters are referred to as "tissue-preferred". Promoters which initiate transcription only in certain tissue are referred to as "tissue-specific". A "cell type" specific promoter primarily drives expression in certain cell types in one or more organs, for example, vascular cells in roots or leaves. An "inducible" promoter is a promoter which is under

25   environmental control. Examples of environmental conditions that may effect transcription by inducible promoters include anaerobic conditions or the presence of light. Tissue-specific, tissue-preferred, cell type specific, and inducible promoters constitute the class of "non-constitutive" promoters. A "constitutive" promoter is a promoter which is active under most environmental conditions.

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A. Inducible Promoters

An inducible promoter is operably linked to a gene for expression in soybean. Optionally, the inducible promoter is operably linked to a nucleotide sequence encoding a signal sequence which is operably linked to a gene for expression in soybean. With

35   an inducible promoter the rate of transcription increases in response to an inducing agent.

Any inducible promoter can be used in the instant invention. *See* Ward *et al. Plant Mol. Biol.*22: 361-366 (1993). Exemplary inducible promoters include, but are not limited to, that from the ACEI system which responds to copper (Mett *et al. PNAS* 90: 4567-4571 (1993)); In2 gene from maize which responds to benzenesulfonamide herbicide safeners (Hershey *et al., Mol. Gen. Genetics* 227: 229-237 (1991) and Gatz *et al., Mol. Gen. Genetics* 243: 32-38 (1994)) or Tet repressor from Tn10 (Gatz *et al., Mol. Gen. Genet.* 227: 229-237 (1991). A particularly preferred inducible promoter is a promoter that responds to an inducing agent to which plants do not normally respond. An exemplary inducible promoter is the inducible promoter from a steroid hormone gene, the transcriptional activity of which is induced by a glucocorticosteroid hormone. Schena *et al., Proc. Natl. Acad. Sci. U.S.A.* 88: 0421 (1991).

B. Constitutive Promoters

A constitutive promoter is operably linked to a gene for expression in soybean or the constitutive promoter is operably linked to a nucleotide sequence encoding a signal sequence which is operably linked to a gene for expression in soybean.

Many different constitutive promoters can be utilized in the instant invention. Exemplary constitutive promoters include, but are not limited to, the promoters from plant viruses such as the 35S promoter from CaMV (Odell *et al., Nature* 313: 810-812 (1985) and the promoters from such genes as rice actin (McElroy *et al., Plant Cell* 2: 163-171 (1990)); ubiquitin (Christensen *et al., Plant Mol. Biol.*12: 619-632 (1989) and Christensen *et al., Plant Mol. Biol.* 18: 675-689 (1992)); pEMU (Last *et al., Theor. Appl. Genet.* 81: 581-588 (1991)); MAS (Velten *et al., EMBO J.* 3: 2723-2730 (1984)) and maize H3 histone (Lepetit *et al., Mol. Gen. Genet.*231: 276-285 (1992) and Atanassova *et al., Plant Journal* 2 (3): 291-300 (1992)).

The ALS promoter, a *Xbal/Nco*I fragment 5' to the *Brassica napus* ALS3 structural gene (or a nucleotide sequence that has substantial sequence similarity to said *Xbal/Nco*I fragment), represents a particularly useful constitutive promoter. See PCT application WO96/30530.

C. Tissue-specific or Tissue-Preferred Promoters

A tissue-specific promoter is operably linked to a gene for expression in soybean. Optionally, the tissue-specific promoter is operably linked to a nucleotide sequence encoding a signal sequence which is operably linked to a gene for expression in soybean. Plants transformed with a gene of interest operably linked to a tissue-

specific promoter produce the protein product of the transgene exclusively, or preferentially, in a specific tissue.

Any tissue-specific or tissue-preferred promoter can be utilized in the instant invention. Exemplary tissue-specific or tissue-preferred promoters include, but are not limited to, a root-preferred promoter- such as that from the phaseolin gene (Murai *et al.,* *Science* 23: 476-482 (1983) and Sengupta-Gopalan *et al., Proc. Natl. Acad. Sci. USA* 82: 3320-3324 (1985)); a leaf-specific and light-induced promoter such as that from *cab* or *rubisco* (Simpson *et al., EMBO J.* 4(11): 2723-2729 (1985) and Timko *et al., Nature* 318: 579-582 (1985)); an anther-specific promoter such as that from *LAT52* (Twell *et al.,* *Mol. Gen. Genet.* 217: 240-245 (1989)); a pollen-specific promoter such as that from *Zm13* (Guerrero *et al., Mol. Gen. Genet.*224: 161-168 (1993)) or a microspore-preferred promoter such as that from *apg* (Twell *et al., Sex. Plant Reprod.* 6: 217-224 (1993).

Signal Sequences For Targeting Proteins to Subcellular Compartments

Transport of protein produced by transgenes to a subcellular compartment such as the chloroplast, vacuole, peroxisome, glyoxysome, cell wall or mitochondrion, or for secretion into the apoplast, is accomplished by means of operably linking the nucleotide sequence encoding a signal sequence to the 5' and/or 3' region of a gene encoding the protein of interest. Targeting sequences at the 5' and/or 3' end of the structural gene may determine, during protein synthesis and processing, where the encoded protein is ultimately compartmentalized.

The presence of a signal sequence directs a polypeptide to either an intracellular organelle or subcellular compartment or for secretion to the apoplast. Many signal sequences are known in the art. See, for example, Becker *et al., Plant Mol. Biol.*20: 49 (1992), Close, P.S., Master's Thesis, Iowa State University (1993), Knox, C., *et al.,* "Structure and Organization of Two Divergent Alpha-Amylase Genes From Barley", *Plant Mol.Biol.* 9: 3-17 (1987), Lerner *et al., Plant Physiol.*91: 124-129 (1989), Fontes *et al.,Plant Cell* 3: 483-496 (1991), Matsuoka *et al., Proc. Natl. Acad. Sci.* 88: 834 (1991), Gould et al., *J. Cell Biol* 108: 1657 (1989), Creissen *et al., Plant J.* 2: 129 (1991), Kalderon, D., Robers, B., Richardson, W., and Smith A., "A short amino acid sequence able to specify nuclear location", *Cell* 39: 499-509 (1984), Stiefel, V., Ruiz-Avila, L., Raz R., Valles M., Gomez J., Pages M., Martinez-Izquierdo J., Ludevid M., Landale J., Nelson T., and Puigdomenech P., "Expression of a maize cell wall hydroxyproline-rich glycoprotein gene in early leaf and root vascular differentiation", *Plant Cell* 2: 785-793 (1990).

Foreign Protein Genes and Agronomic Genes

With transgenic plants according to the present invention, a foreign protein can be produced in commercial quantities. Thus, techniques for the selection and propagation of transformed plants, which are well understood in the art, yield a plurality of transgenic plants which are harvested in a conventional manner, and a foreign protein then can be extracted from a tissue of interest or from total biomass. Protein extraction from plant biomass can be accomplished by known methods which are discussed, for example, by Heney and Orr, *Anal. Biochem.* 114: 92-6 (1981).

According to a preferred embodiment, the transgenic plant provided for commercial production of foreign protein is a soybean plant. In another preferred embodiment, the biomass of interest is seed. For the relatively small number of transgenic plants that show higher levels of expression, a genetic map can be generated, primarily via conventional RFLP, PCR, and SSR analysis, which identifies the approximate chromosomal location of the integrated DNA molecule. For exemplary methodologies in this regard, see Glick and Thompson, METHODS IN PLANT MOLECULAR BIOLOGY AND BIOTECHNOLOGY 269-284 (CRC Press, Boca Raton, 1993). Map information concerning chromosomal location is useful for proprietary protection of a subject transgenic plant. If unauthorized propagation is undertaken and crosses made with other germplasm, the map of the integration region can be compared to similar maps for suspect plants, to determine if the latter have a common parentage with the subject plant. Map comparisons would involve hybridizations, RFLP, PCR, SSR and sequencing, all of which are conventional techniques.

Likewise, by means of the present invention, agronomic genes can be expressed in transformed plants. More particularly, plants can be genetically engineered to express various phenotypes of agronomic interest. Exemplary genes implicated in this regard include, but are not limited to, those categorized below.

1. <u>Genes That Confer Resistance To Pests or Disease And That Encode</u>:

(A) Plant disease resistance genes. Plant defenses are often activated by specific interaction between the product of a disease resistance gene (R) in the plant and the product of a corresponding avirulence (Avr) gene in the pathogen. A plant variety can be transformed with cloned resistance gene to engineer plants that are resistant to specific pathogen strains. See, for example Jones et al., Science 266: 789 (1994) (cloning of the tomato Cf-9 gene for resistance to *Cladosporium fulvum*); Martin et al., Science 262: 1432 (1993) (tomato *Pto* gene for resistance to *Pseudomonas*

syringae pv. tomato encodes a protein kinase); Mindrinos *et al.*, Cell <u>78</u>: 1089 (1994) (*Arabidopsis RSP2* gene for resistance to *Pseudomonas syringae*).

(B)   A gene conferring resistance to a pest, such as soybean cyst nematode. See *e.g.*PCT Application WO96/30517; PCT Application WO93/19181.

(C)   A *Bacillus thuringiensis* protein, a derivative thereof or a synthetic polypeptide modeled thereon. See, for example, Geiser *et al.*, *Gene* <u>48</u>: 109 (1986), who disclose the cloning and nucleotide sequence of a *Bt* δ-endotoxin gene. Moreover, DNA molecules encoding δ-endotoxin genes can be purchased from American Type Culture Collection (Manassas, VA), for example, under ATCC Accession Nos. 40098, 67136, 31995 and 31998.

(D)   A lectin. See, for example, the disclosure by Van Damme *et al.*, *Plant Molec. Biol.* <u>24:</u> 25 (1994), who disclose the nucleotide sequences of several *Clivia miniata* mannose-binding lectin genes.

(E)   A vitamin-binding protein such as avidin. See PCT application US93/06487, the contents of which are hereby incorporated by reference. The application teaches the use of avidin and avidin homologues as larvicides against insect pests.

(F)   An enzyme inhibitor, for example, a protease or proteinase inhibitor or an amylase inhibitor. See, for example, Abe *et al.*, *J. Biol. Chem.* <u>262:</u> 16793 (1987) (nucleotide sequence of rice cysteine proteinase inhibitor), Huub *et al.*, *Plant Molec. Biol.* <u>21</u>: 985 (1993) (nucleotide sequence of cDNA encoding tobacco proteinase inhibitor I), Sumitani *et al.*, *Biosci. Biotech. Biochem.* <u>57</u>: 1243 (1993) (nucleotide sequence of *Streptomyces nitrosporeus* α-amylase inhibitor) and U.S. Patent No. 5,494,813 (Hepher and Atkinson, issued February 27, 1996).

(G)   An insect-specific hormone or pheromone such as an ecdysteroid and juvenile hormone, a variant thereof, a mimetic based thereon, or an antagonist or agonist thereof. See, for example, the disclosure by Hammock et al., Nature <u>344</u>: 458 (1990), of baculovirus expression of cloned juvenile hormone esterase, an inactivator of juvenile hormone.

(H)   An insect-specific peptide or neuropeptide which, upon expression, disrupts the physiology of the affected pest.   For example, see the disclosures of Regan, *J. Biol. Chem.* <u>269</u>: 9 (1994) (expression cloning yields DNA coding for insect diuretic hormone receptor), and Pratt *et al.*, *Biochem. Biophys. Res. Comm.*<u>163</u>: 1243 (1989) (an allostatin is identified in *Diploptera puntata)*. See also U.S. patent No.5,266,317 to Tomalski *et al.*, who disclose genes encoding insect-specific, paralytic neurotoxins.

(I)   An insect-specific venom produced in nature by a snake, a wasp, *etc.* For example, see Pang et al., *Gene* 116: 165 (1992), for disclosure of heterologous expression in plants of a gene coding for a scorpion insectotoxic peptide.

(J)   An enzyme responsible for an hyperaccumulation of a monterpene, a sesquiterpene, a steroid, hydroxamic acid, a phenylpropanoid derivative or another non-protein molecule with insecticidal activity.

(K)   An enzyme involved in the modification, including the post-translational modification, of a biologically active molecule; for example, a glycolytic enzyme, a proteolytic enzyme, a lipolytic enzyme, a nuclease, a cyclase, a transaminase, an esterase, a hydrolase, a phosphatase, a kinase, a phosphorylase, a polymerase, an elastase, a chitinase and a glucanase, whether natural or synthetic. See PCT application WO 93/02197 in the name of Scott et *al.,* which discloses the nucleotide sequence of a callase gene. DNA molecules which contain chitinase-encoding sequences can be obtained, for example, from the ATCC under Accession Nos. 39637 and 67152. See also Kramer *et al., Insect Biochem. Molec. Biol.*23: 691 (1993), who teach the nucleotide sequence of a cDNA encoding tobacco hookworm chitinase, and Kawalleck *et al., Plant Molec. Biol.* 21: 673 (1993), who provide the nucleotide sequence of the parsley *ubi*4-2 polyubiquitin gene.

(L)   A molecule that stimulates signal transduction. For example, see the disclosure by Botella *et al., Plant Molec. Biol.* 24: 757 (1994), of nucleotide sequences for mung bean calmodulin cDNA clones, and Griess *et al., Plant Physiol.*104: 1467 (1994), who provide the nucleotide sequence of a maize calmodulin cDNA clone.

(M)   A hydrophobic moment peptide. See PCT application WO95/16776 (disclosure of peptide derivatives of Tachyplesin which inhibit fungal plant pathogens) and PCT application WO95/18855 (teaches synthetic antimicrobial peptides that confer disease resistance), the respective contents of which are hereby incorporated by reference.

(N)   A membrane permease, a channel former or a channel blocker. For example, see the disclosure by Jaynes et *al., Plant Sci.* 89: 43 (1993), of heterologous expression of a cecropin-β lytic peptide analog to render transgenic tobacco plants resistant to *Pseudomonas solanacearum.*

(O)   A viral-invasive protein or a complex toxin derived therefrom.   For example, the accumulation of viral coat proteins in transformed plant cells imparts resistance to viral infection and/or disease development effected by the virus from which the coat protein gene is derived, as well as by related viruses. See Beachy *et al., Ann. Rev. Phytopathol.*28: 451 (1990).   Coat protein-mediated resistance has been

conferred upon transformed plants against alfalfa mosaic virus, cucumber mosaic virus, tobacco streak virus, potato virus X, potato virus Y, tobacco etch virus, tobacco rattle virus and tobacco mosaic virus. *Id.*

(P)   An insect-specific antibody or an immunotoxin derived therefrom.   Thus, an antibody targeted to a critical metabolic function in the insect gut would inactivate an affected enzyme, killing the insect.   *Cf.* Taylor *et al.*, Abstract #497, SEVENTH INT'L SYMPOSIUM ON MOLECULAR PLANT-MICROBE INTERACTIONS (Edinburgh, Scotland, 1994) (enzymatic inactivation in transgenic tobacco via production of single-chain antibody fragments).

(Q)   A virus-specific antibody. See, for example, Tavladoraki *et al.*, *Nature* 366: 469 (1993), who show that transgenic plants expressing recombinant antibody genes are protected from virus attack.

(R)   A developmental-arrestive protein produced in nature by a pathogen or a parasite. Thus, fungal endo α-1,4-D-polygalacturonases facilitate fungal colonization and plant nutrient release by solubilizing plant cell wall homo-α-1,4-D-galacturonase. See Lamb *et al.*, *Bio/Technology* 10: 1436 (1992).   The cloning and characterization of a gene which encodes a bean endopolygalacturonase-inhibiting protein is described by Toubart *et al.*, *Plant J.* 2: 367 (1992).

(S)   A developmental-arrestive protein produced in nature by a plant.   For example, Logemann *et al.*, *Bio/Technology* 10: 305 (1992), have shown that transgenic plants expressing the barley ribosome-inactivating gene have an increased resistance to fungal disease.

2.   Genes That Confer Resistance To A Herbicide, For Example:

(A)   A herbicide that inhibits the growing point or meristem, such as an imidazalinone or a sulfonylurea. Exemplary genes in this category code for mutant ALS and AHAS enzyme as described, for example, by Lee *et al.*,*EMBO J.* 7: 1241 (1988), and Miki *et al.*, *Theor. Appl.Genet.* 80: 449 (1990), respectively.

(B)   Glyphosate (resistance imparted by mutant 5-enolpyruvl-3-phosphikimate synthase (EPSP) and *aroA* genes, respectively) and other phosphono compounds such as   glufosinate   (phosphinothricin   acetyl   transferase,   PAT)   and   *Streptomyces hygroscopicus* phosphinothricin-acetyl transferase, *bar*, genes), and pyridinoxy or phenoxy propionic acids and cycloshexones (ACCase inhibitor-encoding genes). See, for example, U.S. patent No. 4,940,835 to Shah et al., which discloses the nucleotide sequence of a form of EPSP which can confer glyphosate resistance. A DNA molecule encoding a mutant *aroA* gene can be obtained under ATCC accession No. 39256, and

the nucleotide sequence of the mutant gene is disclosed in U.S. patent No. 4,769,061 to Comai. European patent application No. 0 333 033 to Kumada *et* al. and U.S. patent No. 4,975,374 to Goodman *et al.* disclose nucleotide sequences of glutamine synthetase genes which confer resistance to herbicides such as L-phosphinothricin. The nucleotide sequence of a phosphinothricin-acetyl-transferase gene is provided in European application No. 0 242 246 to Leemans *et al.* De Greef *et al., Bio/Technology* 7: 61 (1989), describe the production of transgenic plants that express chimeric *bar* genes coding for phosphinothricin acetyl transferase activity. Exemplary of genes conferring resistance to phenoxy propionic acids and cycloshexones, such as sethoxydim and haloxyfop, are the *Acc1-S1, Acc1-S2* and *Acc1-S3* genes described by Marshall *et al., Theor. Appl. Genet.* 83: 435 (1992).

(C)   A herbicide that inhibits photosynthesis, such as a triazine *(psbA* and *gs+* genes) and a benzonitrile (nitrilase gene). Przibilla *et al., Plant Cell* 3: 169 (1991), describe the transformation of *Chlamydomonas* with plasmids encoding mutant *psbA* genes.   Nucleotide sequences for nitrilase genes are disclosed in U.S. patent No. 4,810,648 to Stalker, and DNA molecules containing these genes are available under ATCC Accession Nos. 53435, 67441 and 67442. Cloning and expression of DNA coding for a glutathione S-transferase is described by Hayes *et al., Biochem. J.* 285: 173 (1992).

3.   **Genes That Confer Or Contribute To A Value-Added Trait, Such As:**

(A)   Modified fatty acid metabolism, for example, by transforming a plant with an antisense gene of stearoyl-ACP desaturase to increase stearic acid content of the plant. See Knultzon *et al., Proc. Nat'l. Acad. Sci. USA* 89: 2624 (1992).

(B)   Decreased phytate content

(1)   Introduction of a phytase-encoding gene would enhance breakdown of phytate, adding more free phosphate to the transformed plant. For example, see Van Hartingsveldt *et al., Gene* 127: 87 (1993), for a disclosure of the nucleotide sequence of an *Aspergillus niger* phytase gene.

(2)   A gene could be introduced that reduces phytate content.   In maize, this, for example, could be accomplished, by cloning and then reintroducing DNA associated with the single allele which is responsible   for maize mutants characterized by low levels of phytic acid. See Raboy *et al., Maydica* 35: 383 (1990).

(C)   Modified carbohydrate composition effected, for example, by transforming plants with a gene coding for an enzyme that alters the branching pattern of starch. See

Shiroza et al., J. Bacteriol. 170: 810 (1988) (nucleotide sequence of Streptococcus mutans fructosyltransferase gene), Steinmetz et al., Mol. Gen. Genet. 200: 220 (1985) (nucleotide sequence of Bacillus subtilis levansucrase gene), Pen et al., Bio/Technology 10: 292 (1992) (production of transgenic plants that express Bacillus licheniformis α-

5 amylase), Elliot et al., Plant Molec. Biol. 21: 515 (1993) (nucleotide sequences of tomato invertase genes), Søgaard et al., J. Biol. Chem. 268: 22480 (1993) (site-directed mutagenesis of barley α-amylase gene), and Fisher et al., Plant Physiol. 102: 1045 (1993) (maize endosperm starch branching enzyme II).

10 **Methods for Soybean Transformation**

Numerous methods for plant transformation have been developed, including biological and physical, plant transformation protocols. See, for example, Miki et al., "Procedures for Introducing Foreign DNA into Plants" in Methods in Plant Molecular Biology and Biotechnology, Glick, B.R. and Thompson, J.E. Eds. (CRC Press, Inc.,

15 Boca Raton, 1993) pages 67-88. In addition, expression vectors and in vitro culture methods for plant cell or tissue transformation and regeneration of plants are available. See, for example, Gruber et al., "Vectors for Plant Transformation" in Methods in Plant Molecular Biology and Biotechnology, Glick, B.R. and Thompson, J.E. Eds. (CRC Press, Inc., Boca Raton, 1993) pages 89-119.

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A. *Agrobacterium*-mediated Transformation

One method for introducing an expression vector into plants is based on the natural transformation system of Agrobacterium. See, for example, Horsch et al., Science 227: 1229 (1985). A. tumefaciens and A. rhizogenes are plant pathogenic soil

25 bacteria which genetically transform plant cells. The Ti and Ri plasmids of A. tumefaciens and A. rhizogenes, respectively, carry genes responsible for genetic transformation of the plant. See, for example, Kado, C.I., Crit. Rev. Plant. Sci.10: 1 (1991). Descriptions of Agrobacterium vector systems and methods for Agrobacterium-mediated gene transfer are provided by Gruber et al., supra, Miki et al.,

30 supra, and Moloney et al., Plant Cell Reports 8: 238 (1989). See also, U.S. Patent No. 5,563,055, (Townsend and Thomas), issued Oct. 8, 1996.

B. Direct Gene Transfer

Several methods of plant transformation, collectively referred to as direct gene

35 transfer, have been developed as an alternative to Agrobacterium-mediated transformation. A generally applicable method of plant transformation is

microprojectile-mediated transformation wherein DNA is carried on the surface of microprojectiles measuring 1 to 4 μm. The expression vector is introduced into plant tissues with a biolistic device that accelerates the microprojectiles to speeds of 300 to 600 m/s which is sufficient to penetrate plant cell walls and membranes. Sanford *et al.*, *Part. Sci. Technol.* 5: 27 (1987), Sanford, J.C., *Trends Biotech.* 6: 299 (1988), Klein *et al.*, *Bio/Technology* 6: 559-563 (1988), Sanford, J.C., *Physiol Plant* 79: 206 (1990), Klein *et al.*, *Biotechnology* 10: 268 (1992). See also U.S. Patent No. 5,015,580 (Christou, *et al*), issued May 14, 1991; U.S. Patent No. 5,322,783 (Tomes, *et al.*), issued June 21, 1994.

Another method for physical delivery of DNA to plants is sonication of target cells. Zhang *et al.*, *Bio/Technology* 9: 996 (1991). Alternatively, liposome or spheroplast fusion have been used to introduce expression vectors into plants. Deshayes *et al.*, *EMBO J.*, 4: 2731 (1985), Christou *et al.*, *Proc Natl. Acad. Sci. U.S.A.* 84: 3962 (1987). Direct uptake of DNA into protoplasts using CaCl2 precipitation, polyvinyl alcohol or poly-L-ornithine have also been reported. Hain *et al.*, *Mol. Gen. Genet.*199: 161 (1985) and Draper *et al.*, *Plant Cell Physiol.*23: 451 (1982). Electroporation of protoplasts and whole cells and tissues have also been described. Donn *et al.*, In Abstracts of VIIth International Congress on Plant Cell and Tissue Culture IAPTC, A2-38, p 53 (1990); D'Halluin *et al.*, *Plant Cell* 4: 1495-1505 (1992) and Spencer *et al.*, *Plant Mol. Biol.* 24: 51-61 (1994).

Following transformation of soybean target tissues, expression of the above-described selectable marker genes allows for preferential selection of transformed cells, tissues and/or plants, using regeneration and selection methods now well known in the art.

The foregoing methods for transformation would typically be used for producing a transgenic variety. The transgenic variety could then be crossed, with another (non-transformed or transformed) variety, in order to produce a new transgenic variety. Alternatively, a genetic trait which has been engineered into a particular soybean line using the foregoing transformation techniques could be moved into another line using traditional backcrossing techniques that are well known in the plant breeding arts. For example, a backcrossing approach could be used to move an engineered trait from a public, non-elite variety into an elite variety, or from a variety containing a foreign gene in its genome into a variety or varieties which do not contain that gene. As used herein, "crossing" can refer to a simple X by Y cross, or the process of backcrossing, depending on the context.

TISSUE CULTURE OF SOYBEANS

Further reproduction of the variety can occur by tissue culture and regeneration. Tissue culture of various tissues of soybeans and regeneration of plants therefrom is well known and widely published. For example, reference may be had to Komatsuda, T. et al., "Genotype X Sucrose Interactions for Somatic Embryogenesis in Soybean," Crop Sci. 31:333-337 (1991); Stephens, P.A. et al., "Agronomic Evaluation of Tissue-Culture-Derived Soybean Plants," Theor. Appl. Genet. (1991) 82:633-635; Komatsuda, T. et al., "Maturation and Germination of Somatic Embryos as Affected by Sucrose and Plant Growth Regulators in Soybeans Glycine gracilis Skvortz and Glycine max (L.) Merr.," Plant Cell, Tissue and Organ Culture, 28:103-113 (1992); Dhir, S. et al., "Regeneration of Fertile Plants from Protoplasts of Soybean (Glycine max L. Merr.): Genotypic Differences in Culture Response," Plant Cell Reports (1992) 11:285-289; Pandey, P. et al., "Plant Regeneration from Leaf and Hypocotyl Explants of Glycine wightii (W. and A.) VERDC. var. longicauda," Japan J. Breed. 42:1-5 (1992); and Shetty, K., et al., "Stimulation of In Vitro Shoot Organogenesis in Glycine max (Merrill.) by Allantoin and Amides," Plant Science 81:(1992) 245-251; as well as U.S. Patent 5,024,944, issued June 18, 1991 to Collins et al. and U.S. Patent 5,008,200, issued April 16, 1991 to Ranch et al., the disclosures of which are hereby incorporated herein in their entirety by reference. Thus, another aspect of this invention is to provide cells which upon growth and differentiation produce soybean plants having the physiological and morphological characteristics of soybean variety 92B84.

This invention also is directed to methods for producing a soybean plant by crossing a first parent soybean plant with a second parent soybean plant wherein the first or second parent soybean plant is a soybean plant of the variety 92B84. Further, both first and second parent soybean plants can come from the soybean variety 92B84. Thus, any such methods using the soybean variety 92B84 are part of this invention: selfing, backcrosses, hybrid production, crosses to populations, and the like. All plants produced using soybean variety 92B84 as a parent are within the scope of this invention, including those developed from varieties derived from soybean variety 92B84. Advantageously, the soybean variety could be used in crosses with other, different, soybean plants to produce first generation (F_1) soybean hybrid seeds and plants with superior characteristics. The variety of the invention can also be used for transformation where exogenous genes are introduced and expressed by the variety of the invention. Genetic variants created either through traditional breeding methods using variety 92B84 or through transformation of 92B84 by any of a number of protocols known to those of skill in the art are intended to be within the scope of this invention.

As used herein, the term plant includes plant cells, plant protoplasts, plant cell tissue cultures from which soybean plants can be regenerated, plant calli, plant clumps, and plant cells that are intact in plants or parts of plants, such as embryos, pollen, ovules, seed, flowers, pods, leaves, roots, root tips, anthers, and the like.

Industrial Applicability

The seed of soybean variety 92B84, the plant produced from the seed, the hybrid soybean plant produced from the crossing of the variety with any other soybean plant, hybrid seed, and various parts of the hybrid soybean plant can be utilized for human food, livestock feed, and as a raw material in industry.

The soybean is the world's leading source of vegetable oil and protein meal. The oil extracted from soybeans is used for cooking oil, margarine, and salad dressings. Soybean oil is composed of saturated, monounsaturated and polyunsaturated fatty acids. It has a typical composition of 11% palmitic, 4% stearic, 25% oleic, 50% linoleic and 9% linolenic fatty acid content ("Economic Implications of Modified Soybean Traits Summary Report", Iowa Soybean Promotion Board & American Soybean Association Special Report 92S, May 1990). Changes in fatty acid composition for improved oxidative stability and nutrition are constantly sought after. Industrial uses of soybean oil which is subjected to further processing include ingredients for paints, plastics, fibers, detergents, cosmetics, and lubricants. Soybean oil may be split, inter-esterified, sulfurized, epoxidized, polymerized, ethoxylated, or cleaved. Designing and producing soybean oil derivatives with improved functionality, oliochemistry, is a rapidly growing field. The typical mixture of triglycerides is usually split and separated into pure fatty acids, which are then combined with petroleum-derived alcohols or acids, nitrogen, sulfonates, chlorine, or with fatty alcohols derived from fats and oils.

Soybean is also used as a food source for both animals and humans. Soybean is widely used as a source of protein for animal feeds for poultry, swine and cattle. During processing of whole soybeans, the fibrous hull is removed and the oil is extracted. The remaining soybean meal is a combination of carbohydrates and approximately 50% protein.

For human consumption soybean meal is made into soybean flour which is processed to protein concentrates used for meat extenders or specialty pet foods. Production of edible protein ingredients from soybean offers a healthy, less expensive replacement for animal protein in meats as well as dairy-type products.

Performance Examples of 92B84

In the examples that follow, the traits and characteristics of soybean variety 92B84 are given in paired comparison with another soybean variety. The data collected on each soybean variety is presented for key characteristics and traits.

5   The results in Table 2A compare variety 92B84 to another similarly adapted Pioneer soybean variety, 92B62. According to the table, variety 92B84 is significantly higher yielding than variety 92B62. Variety 92B84 further is later to mature with a significantly later absolute maturity and predicted relative maturity score than variety 92B62. Variety 92B84 demonstrates significantly taller plant stature than variety 92B62.

10   The results in Table 2B compare variety 92B84 to another similarly adapted Pioneer soybean variety, 92B71. The table shows that variety 92B84 is significantly higher yielding than variety 92B71. Variety 92B84 further shows a significantly later absolute maturity score and significantly taller plant stature than variety 92B71.

The results in Table 2C compare variety 92B84 to another similarly adapted

15   Pioneer soybean variety, 92B74. The table shows that variety 92B84 is significantly higher yielding than variety 92B74. Variety 92B84 further demonstrates a significantly later absolute maturity score than variety 92B74. Variety 92B84 demonstrates significantly taller plant stature and a significantly higher seed protein content than variety 92B74.

20   The results in Table 2D compare variety 92B84 to another similarly adapted Pioneer soybean variety, 92B75. The table shows that variety 92B84 is significantly higher yielding than variety 92B75. Variety 92B84 further demonstrates significantly taller plant stature than variety 92B75.

The results in Table 2E compare variety 92B84 to another similarly adapted

25   Pioneer soybean variety, 93B01. The table indicates that variety 92B84 is earlier to mature with a significantly earlier absolute maturity score and predicted relative maturity score than variety 93B01. Variety 92B84 demonstrates significantly taller plant stature with a somewhat higher susceptibility to lodging and a significantly superior tolerance to Brown Stem Rot than variety 93B01.

30   The results in Table 2F compare variety 92B84 to another similarly adapted Asgrow soybean variety, AG2602. The table shows that variety 92B84 is significantly higher yielding than variety AG2602. Variety 92B84 further demonstrates a significantly taller plant stature than variety AG2602.

The results in Table 2G compare variety 92B84 to another similarly adapted

35   Pioneer soybean variety, 9281. The table shows that variety 92B84 demonstrates significantly taller plant stature and a significantly higher seed protein content than

variety 9281. Variety 92B84 further exhibits a significantly superior tolerance to Brown Stem Rot than variety 9281.

The results in Table 2H compare variety 92B84 to another similarly adapted Pioneer soybean variety, 92B91. The results indicate that variety 92B84 demonstrates a significantly earlier absolute maturity score than variety 92B91. Variety 92B84 demonstrates significantly shorter plant stature with significantly superior tolerance to plant lodging and a significantly higher seed protein content than variety 92B91. Variety 92B84 also exhibits significantly lower seed oil content than variety 92B91.

TABLE 2A

PAIRED COMPARISON REPORT

VARIETY #1 =92B84
VARIETY #2 =92B62

VAR #	B/A ABS	MAT ABS ABS	PRM MAT	HGT ABS	LDG ABS	PRO ABS	OIL ABS
92B84	55.4	125.4	29	35.9	7.6	43.2	21.3
92B62	51 7	123.2	26	33.8	6.8	43.3	19.5
REPS	41	23	6	14	11	2	2
DIFF	3 7	2.2	3	2.1	0.8	0	1.8
PROB	0.000#	.000#	.000#	.012+	0.108	0.91	0.288

VAR #	S/L B ABS	BSR ABS	FEC L ABS	SDS ABS
92B84	3003 5	9	7.5	7
92B62	3311 9	9	5.3	7.5
REPS	2	2	1	2
DIFF	308 5	0	2.3	0.5
PROB	0.235	1		0.795

*=significant at the 10% level
+=significant at the 5% level
#=significant at the 1% level

TABLE 2B

PAIRED COMPARISON REPORT

VARIETY #1 =92B84
VARIETY #2 =92B71

VAR #	B/A ABS	MAT ABS ABS	PRM MAT	HGT ABS	LDG ABS	PRO ABS	OIL ABS
92B84	52.1	124.8	28	32.8	7.4	40.8	22.7
92B71	46.7	123.9	28	30.5	7.9	40	22.2
REPS	73	36	8	22	12	6	6
DIFF	5.4	0.8	0	2.3	0.5	0.8	0.5
PROB	0.000#	.029+	0.221	.001#	.074*	0.105	0.32

VAR #	S/L B ABS	BSR ABS	SDS ABS
92B84	3264.4	8.5	7.4
92B71	3300.6	8	7.7
REPS	6	6	3
DIFF	36.3	0.5	0.2
PROB	0.711	.076*	0.635

*=significant at the 10% level
+=significant at the 5% level
#=significant at the 1% level

TABLE 2C

PAIRED COMPARISON REPORT

VARIETY #1 =92B84
VARIETY #2 =92B74

VAR #	B/A ABS	MAT ABS ABS	PRM MAT	HGT ABS	LDG ABS	PRO ABS	OIL ABS
92B84	51.8	125.9	28	34.5	7.4	40.8	22.7
92B74	49	124.8	27	30.7	7.3	38.8	22.8
REPS	48	26	3	13	12	6	6
DIFF	2.7	1.1	1	3.8	0.1	2	0.1
PROB	0.000#	.004#	0.104	.000#	0.6	.002#	0.567

VAR #	S/L B ABS	BSR ABS	SDS ABS
92B84	3264.4	9	6.7
92B74	3305.5	3.5	9
REPS	6	2	2
DIFF	41.1	5.5	2.3
PROB	0.65	0.272	.090*

*=significant at the 10% level
+=significant at the 5% level
#=significant at the 1% level

TABLE 2D

PAIRED COMPARISON REPORT

VARIETY #1 =92B84
VARIETY #2 =92B75

VAR #	B/A ABS	MAT ABS ABS	PRM MAT	HGT ABS	LDG ABS	PRO ABS	OIL ABS
92B84	52.2	124.8	28	32.8	7.4	40.7	22.8
92B75	49.7	124.3	28	30.4	8.2	40.4	23.2
REPS	72	36	8	22	12	5	5
DIFF	2.5	0.5	0	2.4	0.8	0.3	0.4
PROB	0.001#	0.266	0.97	.000#	.054*	0.662	0.286

VAR #	S/L B ABS	BSR ABS	SDS ABS
92B84	3250.2	8.5	7.4
92B75	3084	8	5.2
REPS	5	6	3
DIFF	166.2	0.5	2.2
PROB	0.225	.076*	0.362

*=significant at the 10% level
+=significant at the 5% level
#=significant at the 1% level

TABLE 2E

PAIRED COMPARISON REPORT

VARIETY #1 =92B84
VARIETY #2 =93B01

VAR #	B/A ABS	MAT ABS ABS	PRM MAT	HGT ABS	LDG ABS	PRO ABS	OIL ABS
92B84	52.1	124.8	28	32.8	7.4	40.7	22.8
93B01	51.4	126.7	30	30.1	8.2	40	22.9
REPS	73	36	8	22	12	5	5
DIFF	0.8	2	2	2.8	0.8	0.7	0.1
PROB	0.302	.000#	.002#	.000#	.036+	.078*	0.659

VAR #	S/L B ABS	BSR ABS	FEC L ABS	SDS ABS
92B84	3250 2	8.5	5	7.4
93B01	3521.3	6.3	6	8
REPS	5	6	1	3
DIFF	271.1	2.2	1	0.6
PROB	0.148	.010+		0.423

*=significant at the 10% level
+=significant at the 5% level
#=significant at the 1% level

TABLE 2F

PAIRED COMPARISON REPORT

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VARIETY #1 =92B84
VARIETY #2 =AG2602

VAR #	B/A ABS	MAT ABS ABS	PRM MAT	HGT ABS	LDG ABS	BSR ABS	SDS ABS
92B84	52.5	125.9	28	35	7.3	9	7
AG2602	49.2	125.3	27	32.1	7.9	9	7
REPS	21	12	1	5	5	1	1
DIFF	3.3	0.6	1	2.9	0.6	0	0
PROB	0.007#	0.297		.008#	0.178		

*=significant at the 10% level
+=significant at the 5% level
#=significant at the 1% level

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TABLE 2G

PAIRED COMPARISON REPORT

VARIETY #1 =92B84
VARIETY #2 =9281

VAR #	B/A ABS	MAT ABS ABS	PRM MAT	HGT ABS	LDG ABS	PRO ABS	OIL ABS
92B84	54 3	125.4	29	35.4	7.6	41.7	22.4
9281	54 9	125.1	28	32	7.9	40.5	23.2
REPS	47	25	7	17	13	8	8
DIFF	0 6	0.3	1	3.4	0.3	1.2	0.8
PROB	0.484	0.425	0.102	.000#	0.337	.004#	.061*

VAR #	S/L B ABS	BSR ABS	FEC L ABS	SDS ABS
92B84	3155 3	9	6.3	6.7
9281	3069 7	7	5.5	7.5
REPS	8	2	2	2
DIFF	85 6	2	0.8	0.8
PROB	0.262	.000#	0.205	0.126

*=significant at the 10% level
+=significant at the 5% level
#=significant at the 1% level

TABLE 2H

PAIRED COMPARISON REPORT

VARIETY #1 =92B84
VARIETY #2 =92B91

VAR #	B/A ABS	MAT ABS ABS	PRM MAT	HGT ABS	LDG ABS	PRO ABS	OIL ABS
92B84	54 3	125.4	29	35 4	7.6	41.6	22.4
92B91	52 3	126.4	30	39 6	4.3	39.2	22.9
REPS	47	25	7	17	13	9	9
DIFF	2	1.1	1	4 3	3.3	2.4	0.6
PROB	0.104	.025+	0.162	.000#	.000#	.000#	.025+

VAR #	S/L B ABS	BSR ABS	FEC L ABS	SDS ABS
92B84	3175 3	9	6.6	6 7
92B91	3254 5	9	6.7	8 2
REPS	9	2	2	2
DIFF	79 2	0	0.1	1 5
PROB	0.478	1	0.91	0.205

*=significant at the 10% level
+=significant at the 5% level
#=significant at the 1% level

Deposits

A deposit of the seed of soybean variety 92B84 is and has been maintained by Pioneer Hi-Bred International, Inc., 800 Capital Square, 400 Locust Street, Des Moines, Iowa 50309-2340, since prior to the filing date of this application. Access to this deposit will be available during the pendency of the application to the Commissioner of Patents and Trademarks and persons determined by the Commissioner to be entitled thereto upon request. Upon allowance of any claims in the application, the Applicant(s) will make available to the public without restriction a deposit of at least 2500 seeds of variety 92B84 with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209. The seeds deposited with the ATCC will be taken from the same deposit maintained at Pioneer Hi-Bred and described above. Additionally, Applicant(s) will meet all the requirements of 37 C.F.R. §1.801 - 1.809, including providing an indication of the viability of the sample when the deposit is made. This deposit of Soybean variety 92B84 will be maintained in the ATCC Depository, which is a public depository, for a period of 30 years, or 5 years after the most recent request, or for the enforceable life of the patent, whichever is longer, and will be replaced if it ever becomes nonviable during that period. Applicant will impose no restrictions on the availability of the deposited material from the ATCC; however, Applicant has no authority to waive any restrictions imposed by law on the transfer of biological material or its transportation in commerce. Applicant does not waive any infringement of its rights granted under this patent or under the Plant Variety Protection Act (7 USC 2321 et seq.).

The foregoing invention has been described in detail by way of illustration and example for purposes of clarity and understanding. However, it will be obvious that certain changes and modifications such as single gene modifications and mutations, somoclonal variants, variant individuals selected from large populations of the plants of the instant variety and the like may be practiced within the scope of the invention, as limited only by the scope of the appended claims.